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Abstract: To date, only few studies have assessed oral immunotherapy (OIT) for wheat allergy and often describe severe adverse reactions during therapy. We developed partially hydrolyzed wheat-based cereals (pHC), which were used in a multicenter, open-label, OIT pilot study, in immunoglobulin E-mediated wheat allergy children (NCT01332084). The primary objective of the study was to test whether wheat allergic patients tolerate pHC and primary end point was the presence or not of immediate adverse reactions to pHC during the 1-day initial escalation phase (stepwise increased doses of pHC), with evaluation of the maximum dose tolerated. Of the 9 patients enrolled in the trial, 4 discontinued OIT because of mild to severe reactions at the initial escalation phase. The 5 patients who passed the escalation phase consumed pHC daily for 1 to 6 months. One of these patients withdrew due to noncompliance, whereas the 4 others completed the study and successfully passed the wheat challenge test at the end of the study. About 60% of the adverse events were unrelated to the study product. Our study provides preliminary evidence that pHC is tolerated by a subset of wheat allergic patients. Further studies are warranted to test its efficacy as a potential therapeutic option for wheat allergic patients.

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Oral Immunotherapy With Partially Hydrolyzed Wheat-Based Cereals: A Pilot Study

Roger Lauener^{1,2}, Philippe A Eigenmann³, Jacqueline Wassenberg⁴, Andreas Jung⁵, Sandra Denery-Papini⁶, Sigrid Sjölander⁷, Sophie Pecquet⁸, Rodolphe Fritsché⁹, Adrian Zuercher¹⁰, Antoine Wermeille⁹, Massimo Fontanesi¹¹, Annick Mercenier⁹, Yvonne M Vissers⁹ and Sophie Nutten⁹

¹St. Gallen, Children's Hospital of Eastern Switzerland, St. Gallen, Switzerland. ²Christine Kühne Center for Allergy Research and Education (CK-CARE), Davos, Switzerland. ³Pediatric Allergy Unit, Geneva University Hospital, Geneva, Switzerland. ⁴Division of Immunology and Allergy, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland. ⁵Department of Pulmonology, University Children's Hospital Zurich, Zurich, Switzerland. ⁶UR 1268 Biopolymers Interactions Assemblies, INRA, Nantes, France. ⁷ImmunoDiagnostics, Thermo Fisher Scientific, Uppsala, Sweden. ⁸Nestlé Nutrition Institute, Vevey, Switzerland. ⁹Allergy Group, Nutrition and Health Research, Nestlé Research Center, Lausanne, Switzerland. ¹⁰CSL Behring AG, Research, Bern, Switzerland. ¹¹Cereals and Cocoa Beverages Department, Nestlé Product Technology Centre Orbe, Orbe, Switzerland.

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ABSTRACT: To date, only few studies have assessed oral immunotherapy (OIT) for wheat allergy and often describe severe adverse reactions during therapy. We developed partially hydrolyzed wheat-based cereals (pHC), which were used in a multicenter, open-label, OIT pilot study, in immunoglobulin E-mediated wheat allergy children (NCT01332084). The primary objective of the study was to test whether wheat allergic patients tolerate pHC and primary end point was the presence or not of immediate adverse reactions to pHC during the 1-day initial escalation phase (stepwise increased doses of pHC), with evaluation of the maximum dose tolerated. Of the 9 patients enrolled in the trial, 4 discontinued OIT because of mild to severe reactions at the initial escalation phase. The 5 patients who passed the escalation phase consumed pHC daily for 1 to 6 months. One of these patients withdrew due to noncompliance, whereas the 4 others completed the study and successfully passed the wheat challenge test at the end of the study. About 60% of the adverse events were unrelated to the study product. Our study provides preliminary evidence that pHC is tolerated by a subset of wheat allergic patients. Further studies are warranted to test its efficacy as a potential therapeutic option for wheat allergic patients.

KEYWORDS: wheat, allergy, oral immunotherapy, hydrolysate

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CORRESPONDING AUTHOR: Sophie Nutten, Allergy Group, Nutrition and Health Research, Nestlé Research Center, PO Box 44, Lausanne CH-1000, Switzerland. Email: sophie.nutten@rdls.nestle.com

Introduction

Wheat is the most widely cultivated cereal and is extensively used in food products. Wheat allergy is diagnosed, based on oral food challenge (OFC), in 0.48% in children in a recently published paper.^{1,2} Although the standard therapy for food allergy is strict avoidance of the incriminated food, this approach severely affects the patient's quality of life with a persisting risk of an accidental allergic reaction through inadvertent contact.³ Oral immunotherapy (OIT), performed by oral exposure to increasing doses of the offending food substance, is currently seen as a promising option for the treatment of food allergies. However, one major drawback of OIT using standard, allergenic food products, is the possibility of severe allergic reactions, which are observed mainly during the initial escalation phase.^{3,4} We aimed at designing a less allergenic but still immunogenic wheat-based product to be used in OIT. Wheat allergic patients should tolerate this product better than intact whey proteins, hence reducing the number and severity of

adverse events observed during the initial escalation phase of an OIT trial and allowing to reduce the timing needed to reach the maintenance dose and as a consequence the number of days spent in hospital.

We therefore developed low-allergenic wheat-based cereals, by partial enzymatic hydrolysis of wheat flour (Nestlé Product Technology Centre Orbe, Orbe, Switzerland), which were used in a multicenter, open-label, OIT pilot study in children diagnosed with immunoglobulin E (IgE)-mediated wheat allergy. The study was conducted in Switzerland between 2011 and 2012.

Materials and Methods

The study product consisted of partially hydrolyzed wheat proteins (8.8 g equivalent protein/100 g powder), carbohydrates, essential amino acids, fats, and vitamins, produced at the Nestlé Product Technology Centre Orbe.



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V0 Screening	V1 Initial escalation phase of pHC by challenging sequential doses	Maintenance phase Maximum dose tolerated at V1 administered in one serving daily	Final visit Challenge test with regular wheat
1 day (in hospital)	1 day (in hospital)	1-6 months (at home)	1 day (in hospital)
Positive SPT and/or wheat IgE & positive challenge or clear history of allergy	<ul style="list-style-type: none"> T0': 0.2g T30': 2g T60': 8g T90': 25g 		
Informed consent	Physical examination, anthropometry, concomitant diseases	Monthly phone calls to monitor adverse reactions and compliance	Physical examination, anthropometry, adverse reactions and compliance
Blood sample ^a	Blood sample		Blood sample

Figure 1. Study protocol. IgE indicates immunoglobulin E; SPT, skin prick test.

^aOnly for individuals without preliminary results for specific IgE sensitization to wheat ≤ 1 year before screening.

Hydrolysis of wheat proteins was assessed by size-exclusion chromatography performed by resuspending wheat flour or partially hydrolyzed wheat-based cereals (pHC) in 2% (v/v) acetonitrile/0.1% (v/v) formic acid and injection of the soluble fraction on a Superdex Peptide 10/300 GL column. Allergenicity of the pHC was assessed by gliadin quantification (commercially available enzyme-linked immunosorbent assay [ELISA] kit optimized for raw and process foods matrices [181GD; Morinaga, Tokyo, Japan]), recognition by antibodies of specific wheat components (indirect ELISA) and by in vitro cross-linking activity, assessed by degranulation of rat basophilic leukemia cells (RBLs).

Subjects (2-12 years) with positive skin prick test (SPT) or positive specific IgE and immediate positive reaction to wheat (confirmed by a physician supervised standard OFC performed ≤ 1 year before enrollment) or unequivocal history of an immediate reaction to wheat suggestive of allergy were recruited. Skin prick tests were performed using a wheat extract from Allergopharma (Reinbek, Germany) and histamine control from ALK-Abello (Hørsholm, Denmark). The wheal size was calculated as $(D + d)/2$, in which "D" is the longest diameter of the wheal, and "d" is the longest diameter orthogonal to D. A cutoff of ≥ 3 mm was used for a positive SPT. Wheat-specific IgE levels were determined using ImmunoCAP (Thermo Fisher, Uppsala, Sweden) according to the instructions of the manufacturer. Positive IgE levels were considered if >0.35 kU/L. Open food challenges were performed with wheat bread or wheat porridge in 2 centers and with commercial cooked Ebly (<https://www.ebly.com/>) in 1 center, following the routinely used protocol in the respective centers.

The OIT protocol included a 1-day initial escalation phase with pHC (maximum dose: 25 g, maximum cumulative dose: 35.2 g), followed by a 1- to 6-month maintenance phase (Figure 1).

For the escalation phase, the product was prepared by reconstitution with water. Partially hydrolyzed wheat-based cereals were administered every 30 minutes with increasing doses as

follows: step 1: 0.2 g (0.018 g equivalent protein), step 2: 2 g (0.18 g equivalent protein), step 3: 8 g (0.7 g equivalent protein), step 4: 25 g (2.2 g equivalent protein), for a total amount of 35.2 g (3.1 g equivalent protein). Both objective and subjective symptoms were recorded at each step.

Subjects having tolerated the maximum dose of pHC (25 g) entered the maintenance phase; subjects having an immediate allergic reaction to the study product during the first day were proposed to come back the day after to do a second escalation phase starting with the maximum dose tolerated during the first escalation phase. If the subject had a reaction with a cumulative dose lower than 10.2 g, the subject was withdrawn. If the maximum cumulative dose tolerated was between 10.2 and 35.2 g, the subject continued the protocol at home with the maximum dose of pHC given as the maintenance dose.

For the maintenance phase, the product was dissolved in water or mixed with other food well tolerated by the child. Parents were asked not to heat up the study product and to give it to the child in one serve every day until the final visit. Maintenance phase duration was encouraged up to 6 months, but shorter timing (1 month minimum) was accepted in case patients would like to stop for nontolerance, for example.

The primary end point was the presence or not of immediate adverse reactions to pHC during the initial escalation phase, with evaluation of the maximum dose tolerated. The secondary end points were compliance with product intake, morbidity as assessed by the frequency of adverse events, immunologic parameters (antibody response to wheat), and allergic reactions during the regular open food challenge test at final visit. The open food challenge test was conducted using Ebly, a wheat-based product commercialized in European countries such as Switzerland. Every 30 minutes, the product was administered with increasing doses as follows: step 1: 0.66 g Ebly (0.03 g protein), step 2: 2.2 g Ebly (0.1 g protein), step 3: 6.6 g Ebly (0.3 g protein), step 4: 22 g Ebly (1 g protein), and step 5: 66 g Ebly (3 g protein) (total amount of 4.43 g protein). Any occurrence of objective or

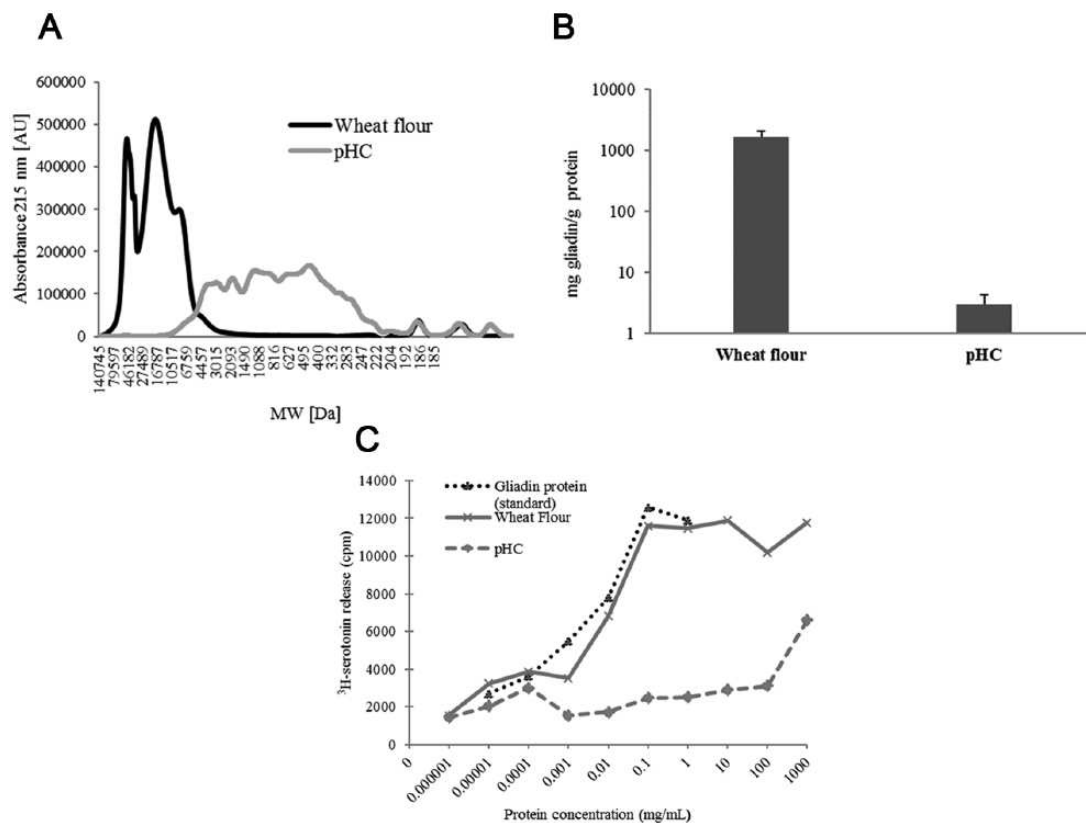


Figure 2. Peptide profile and allergenicity assessment of pHC compared with wheat. (A) Size-exclusion chromatography, (B) gliadin quantification using a commercial enzyme-linked immunosorbent assay kit and (C) in vitro ³H-serotonin release from anti-gliadin IgE-bearing rat basophilic leukemia cells (RBL-2H3 cell line) exposed to pHC, wheat flour, or gliadin. MW indicates molecular weight; pHC, partially hydrolyzed wheat-based cereals.

persistent (45 minutes) subjective symptoms was considered as a positive result to the challenge test.

No inferential analysis was attempted for this trial, and therefore, all the analyses conducted were descriptive. Clinical research protocol was approved by the Ethical Review Board of Lausanne, CH (*Commission cantonale d'éthique de la recherche sur l'être humain*, Ref. 297/10); Geneva, CH (*Commission centrale d'éthique de la recherche sur l'être humain*, Ref. 10-260); and Zürich, CH (*Kantonale ethikkommission Zürich*, Ref. 2010-0218/2). All parents of enrolled subjects provided informed consent before the start of the study.

Results

Hydrolysis of wheat proteins was assessed by size-exclusion chromatography (Figure 2A). The low allergen content of pHC was shown by the level of gliadin, being the most allergenic wheat protein, (3.0 ± 1.3 mg/g protein), which was more than 500times lower than gliadin levels in wheat flour (1655.9 ± 417.2 mg/g protein) (Figure 2B). The mediator release assays of RBLs showed a highly reduced gliadin-induced degranulation capacity ($>10^5$ -fold reduction) of pHC compared with both gliadin and wheat flour (Figure 2C). Epitopes from γ - and ω -gliadins (monoclonal antibodies used were directed against repeated and N-terminal sequences) and from low- and high-molecular-weight glutenins were not detectable in the pHC, whereas epitopes from the α -gliadins (C-terminal sequence) appeared to be more clearly recognized

in the pHC than in the wheat extract. Finally, epitopes from lipid transfer protein (LTP) and α -amylase inhibitors were decreased in pHC but still present (Figure 3).

Nine children were enrolled in the trial (mean age: 7.2 ± 3.23 years, 8 boys) (Table 1). Allergy assessment was done at inclusion; respiratory and food allergies other than wheat allergy were recorded (Table 1). Only 2 of the 9 patients described in the study were allergic to wheat only, 3 had respiratory allergy (to dust mite, pollen, and/or cat dander), and 6 had allergy to other foods than wheat (including milk, egg, and nuts).

All had a clear history of IgE-mediated wheat allergy. An immediate positive reaction to wheat by OFC was confirmed in 6 of the 9 subjects within a year before enrollment; dose of the wheat-based product used for the challenge varied a lot between patients (Table 1) and no relation could be observed with the tolerance or not of the pHC during the initial escalation phase. The 3 other patients presented high levels of wheat-specific IgE (89, >100 , and >100 kU/L, respectively) together with a convincing history at inclusion. Four (44%) showed allergic reactions after ingestion of pHC at the initial escalation phase and OIT was not continued (Table 1). Allergic reactions were typical manifestations of mild to severe wheat allergy (Table 1) and were successfully treated with antihistamines; no patient needed adrenaline. The 5 other patients tolerated the full dose of 25 g. Patients who passed the initial escalation phase tended to display lower wheat IgE levels at

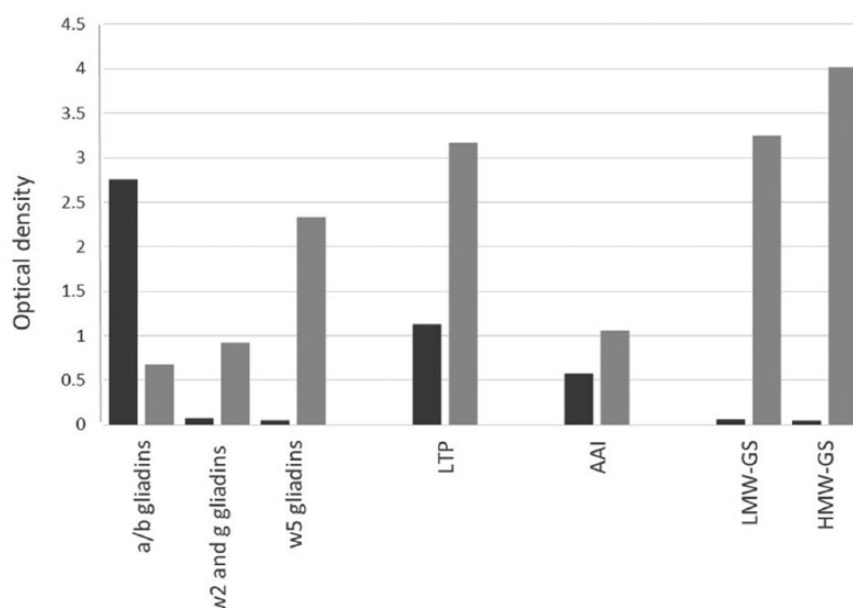


Figure 3. Recognition of specific wheat components in wheat flour and pHC. Intensity of response (expressed in optical density unit) in antigen-coated plate indirect enzyme-linked immunosorbent assay kit, using antibodies specific for several wheat allergens: α/β -gliadins, ω -2 and γ -gliadins, ω -5 gliadins, LTP (lipid transfer protein), AAI (α -amylase inhibitors), HMW-GS (high-molecular-weight glutenins), and LMW-GS (low-molecular-weight glutenins). Black and gray bars correspond to pHC and wheat flour, respectively.

Table 1. Clinical features and compliance data.

PATIENT	AGE (Y)/SEX	OTHER ALLERGIES THAN WHEAT ALLERGY ^a	MAXIMUM TOLERATED DOSE (FOOD CHALLENGE PRIOR TRIAL)	HIGHEST pHc DOSE TOLERATED DURING INITIAL ESCALATION PHASE	HIGHEST INTENSITY ^b OF THE ALLERGIC REACTIONS ^c DURING INITIAL ESCALATION PHASE	PATIENT COMPLIANCE	
						DAYS IN TRIAL	DAYS OFF THE PRODUCT (%)
Patients tolerating pHc							
1	7.4/m	Mi, Fi, Nu	45 g wheat bread	25	nr	185	4 (2.2)
2	10.7/m	Fi, Po	20 g wheat porridge	25	nr	196	0.5 (0.3)
3	11/m	Mi, Eg, Po, DM, Ca	nd	25	U2	186	1 (0.5)
4	2.8/m	None	2.8 g wheat bread	25	nr	155	2 (1.3)
*	4.4/m	Mi, Eg, Nu, Pe, Ba, DM, Po	30 mg wheat bread	25	nr	83	54 (65.1)
Patients not tolerating pHc							
5	4.6/m	Eg	nd	2	I1, C1, W1	1	0
6	8.5/f	Po	nd	0.2	U2, R2, W3, H3, I1, B3	1	0
7	4.7/m	None	2.8 g wheat bread	2	V1, U2, S2, A2	1	0
8	11.1/m	Eg	58.5 g Ebly	8	I2, P1, T1	1	0

Abbreviations: f, female; LTP, lipid transfer protein; m, male; nd, not done; nr, no reaction; pHC, partially hydrolyzed wheat-based cereals.

^aAllergy to Ba: banana, Ca: cat, DM: dust mite, Eg: egg, Fi: fish, Mi: milk, Nu: nuts, Pe: peanut, Po: pollens.

^bIntensity of allergic reactions: (1) mild, (2) moderate, and (3) severe.

^cAllergic reactions: U: urticaria, I: itching, C: coughing, W: wheezing, R: rhinitis, H: hoarseness, B: breathlessness, V: vomiting, S: sneezing, A: angio-edema, P: abdominal pain, T: chest tightness.

*Withdrew.

inclusion in the trial than those who failed the tolerance test (Table 1). High levels of wheat IgE (≥ 100 kU/L), and possibly elevated levels of IgE to gliadin, appeared to be predictors of a

clinical reaction to pHC. Immunoglobulin E to ω -5 gliadin and wheat LTP both appeared to be less predictive of clinical reactivity (Table 2). Of the 5 patients who tolerated the study

Table 2. Specific IgE levels.

PATIENT	IgE LEVELS TO WHEAT PROTEINS (MEASURED BY IMMUNOCAP, KU/L)			
	WHEAT	GLIADIN	w-5 GLIADIN	WHEAT LTP
Patients tolerating pHC				
1	3.34	0.78	<0.35	1.49
2	86.9	10.3	0.65	4.18
3	89	24.7	2.08	<0.35
4	0.41	<0.35	<0.35	<0.35
*	15.85	3.43	<0.35	1.13
Patients not tolerating pHC				
5	>100	>100	5.51	35.4
6	>100	>100	nm	nm
7	100	nm	nm	nm
8	>100	22	0.38	0.62

Abbreviations: LTP, lipid transfer protein; nm, not measured; pHC, partially hydrolyzed wheat-based cereals.

*Withdrew.

product, 4 completed the trial, with strict compliance with product intake (155–196 days of product intake, corresponding to >97%; Table 1). One patient was withdrawn due to noncompliance (83 days of product intake of the 137 days in the study, corresponding to 34.9%; Table 1). Most (60%) of the adverse events that occurred were unrelated to the study product, 16% were unlikely to be related, and 24% were probably related. Only 2 of the 5 patients who tolerated pHC and continued the trial through the maintenance phase at a dose of 25 g pHC had adverse events probably related to the study product. One patient had severe abdominal pain for 2 days (no medication prescribed) and 2 moderate eczema episodes (duration of 11 and 8 days, respectively). Eczema was treated with oral and topical antihistamines and a topical calcineurin inhibitor. The other patient once had moderate urticaria lasting 1 day and was treated with oral antihistamines. No patient needed adrenaline. The 4 children who completed the study all successfully passed the wheat challenge test at the end of the study (commercially available wheat-based product [Ebly]) and could consume 60 to 66 g of pHC (7.3–9.0 g equivalent hydrolyzed protein).

Discussion

In this pilot study, although nearly half of the study population showed allergic reactions to the low-allergenic pHC during the initial challenge at V1 (primary outcome), the patients who completed the trial all successfully passed the wheat challenge test.

Characterization of the pHC showed that despite the notable reduction in gliadin content and gliadin-induced degranulation capacity, certain wheat components remained sufficiently

antigenic, and this may have caused the allergic reactions in the patients during the initial escalation phase. For this group of patients, such an initial day escalation with pHC might not be the best option for starting OIT. Characterization data of the pHC combined with the sensitization profile of patients could help selecting patients tolerating pHC and therefore eligible for OIT using pHC.

Another criteria to help predicting which patient would tolerate or not the pHC would be to relate it to the results of a wheat challenge prior to enrollment. However, due to the non-harmonized protocols used to perform the OFCs in the different centers before the trial and the few numbers of patients included, we cannot draw any conclusion from this trial.

The initial escalation phase for pHC conducted at V1 may have exerted similar effects to those of an ultra-rush induction phase for OIT, and this might have contributed to the beneficial effects observed. The advantage of using pHC is the short time frame (1 day) required to reach the maintenance dose as compared with the up dosing procedure used in most OIT protocols, which can take up to several months including several days in a medical facility.^{5,6} In the context of this pilot trial, we did not assess whether the patients who reacted to pHC at V1 might have been able to reach the maintenance dose with more gradual increases in the dose administered.

Finally, as we did not compare the outcome of the initial escalation phase between intact wheat and pHC in similarly sensitized group of patients, we cannot answer the hypothesis that pHC is a better option for OIT than normal wheat flour.

One of the strengths of our study is the promising results regarding efficacy, yet to be confirmed in a larger placebo-controlled study. However, we believe that the chances of natural outgrow were very low in our patients. Mean age of the children at recruitment was 7.2 ± 3.23 years, which corresponds to the median age for resolution of wheat allergy described in literature,⁷ and the 4 subjects who tolerated wheat after the OIT were 7.4, 10.7-, 11-, and 2.8-year old at enrollment. In addition, some patients were highly allergic as shown by the low dose of wheat inducing allergic reaction during the wheat challenge performed within 1-year prior enrollment into the study.

One advantage of using pHC would be safety. No serious adverse event necessitating the use of epinephrine happened during the 1-day initial escalation phase. During the maintenance phase, only 2 of the 5 patients had adverse events probably related to the study product.

Other promising wheat OIT studies have been published recently using different approaches to propose safer and efficacious treatment. Khayatadeh et al⁸ reported a study using bread in rush OIT ($n=8$ anaphylactic patients, 5–7 years old) and outpatient protocol ($n=5$ nonanaphylactic patients, 5–19 years old). Despite similar limitations as our study (low number of patients, no placebo group, no food challenge at inclusion), all patients tolerated 5.2 g of wheat protein at the end the maintenance phase. All subjects enrolled successfully

completed the build-up phase (several days or several weeks depending on the protocol) as compared with 56% in our study, suggesting that despite pHC is less allergenic than intact wheat protein, 1-day escalation phase was possibly too short for some of the patients. Severe symptoms during both build-up and maintenance phase needed the administration of epinephrine (in the anaphylactic group) and/or short-acting β -agonists in both groups in the study by Khayatzaadeh et al. On the contrary, Rodriguez del Rio et al.⁶ reported only mild adverse reactions during the dosing-up phase in 2 of the 6 patients, and only 1 of the 5 patients experienced a reaction during maintenance phase (generalized urticaria induced by exercise immediately after intake), whereas in our study, no rescue medication was needed. In the study by Rodriguez del Rio, patients were prescribed daily oral antihistamine during the up dosing phase and the treatment was tapered over the first week of the maintenance phase, which could explain the only mild adverse events despite the use of intact protein in the protocol. Finally, a Japanese study using boiled udon as an alternative to intact wheat protein and combining a 5-day rush and a several months build-up phase before the maintenance phase reported 89% of success for desensitization and 61.1% of sustained unresponsiveness in patients with wheat-induced anaphylaxis.⁹ Despite the need to use intramuscular adrenaline to treat adverse reactions in a few patients, this study represents a promising alternative to the use of intact wheat protein for OIT.

In conclusion, our study does provide preliminary evidence that OIT using a low-allergenic pHC product is applicable in a “home setting” and thus a promising therapeutic option for wheat allergic patients who tolerate pHC. The limitations of our study inherent to a pilot study are the small sample size, which clearly limits the interpretation or generalization of the findings, and the lack of a placebo-controlled group.

Results have to be confirmed in a clinical setting including higher numbers of subjects and a placebo group. It also has to be confirmed whether the subpopulation that tolerates pHC

and thus would profit from OIT could be identified by wheat-specific IgE levels below 100 kU/L.

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Author Contributions

RL, PAE, JW, RF, AZ, AW, MF, AM, and SN designed the study/study product. SD and SS conducted the laboratory studies. RL, PAE, JW, and AJ conducted the clinical part of the study. RL, PAE, JW, SD, SP, YMV, and SN interpreted the data and wrote the paper. All authors have read and approved the final manuscript.

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